

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning at page 5, line 7 with the following amended paragraph:

Figure 5: IL-1 receptor binding assay indicates that TNFRII-Fc-IL-1ra chimera binds human IL-1 receptor with higher affinity than marketed non-glycosylated IL-1ra (KINERET Kineret).

Please replace the paragraph beginning at page 5, line 14 with the following amended paragraph:

Figure 8: Cell-based TNF alpha neutralization test indicates that similar to marketed TNFRII-Fc (ENBREL Enbrel), TNFRII-Fc-IL-1ra chimera neutralizes TNF alpha's killing activity on L979 cells.

Please replace the paragraph beginning at page 5, line 17 with the following amended paragraph:

Figure 9: Cell-based IL-1 neutralization test indicates that both marketed IL-1ra (KINERET Kineret) and TNFRII-Fc-IL-1ra chimera neutralize IL-1's biological activity on D10 cell proliferation. As expected, glycosylated IL-1ra has lower in vitro activity than E-coli made non-glycosylated IL-1ra (KINERET Kineret).

Please replace the paragraph beginning at page 26, line 29 with the following amended paragraph:

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CREMOPHOR Cremophor EL (BASF; Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and

liquid polyethyleneglycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Please replace the paragraph beginning at page 37, line 15 with the following amended paragraph:

For IL-1 receptor binding assay, recombinant human IL-1 receptor extracellular domain was first produced in house using a mammalian host. TNFRII-Fc-IL-1ra, negative control TNFRII-Fc and positive control IL-1ra (KINERET Kineret) have been coated to 96-well plate. IL-1 receptor is then incubated at 37° C for binding. The binding is detected using rabbit anti human IL-1 receptor extracellular domain antibodies, followed by goat anti rabbit IgG conjugated with HRP. Figure 5 shows that both TNFRII-Fc-IL-1ra and IL-1ra (KINERET Kineret) bind to IL-1 receptor, and that TNFRII-Fc (ENBREL Enbrel) does not bind.

Please replace the paragraph beginning at page 37, line 23 with the following amended paragraph:

For TNF alpha binding assay, recombinant TNF alpha has been coated on a 96 well plate. TNFRII-Fc-IL-1ra, positive control TNFRII-Fc (ENBREL Enbrel) and negative control Tie2 (ANG-I receptor extracellular domain)-Fc was then incubated at 37°C for binding. The binding was detected by anti human IgG Fc antibodies conjugated with HRP. Figures 6[,] and 7 show that both TNFRII-Fc-IL-1ra chimera and TNFRII-Fc bind to TNF alpha, and that negative control Tie2-Fc does not bind to TNF alpha.

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Please replace the paragraph beginning at page 38, line 4 with the following amended paragraph:

Bioassay and functional testing of TNFRII-Fc-IL-1ra, control TNFRII-Fc (ENBREL Enbrel), and control IL-1ra (KINERET Kineret)

Please replace the paragraph beginning at page 38, line 6 with the following amended paragraph:

For cell-based IL-1 neutralization assay, IL-1 dependent D10 cells (ATCC) have been employed to test the blocking activity of IL-1ra (KINERET Kineret) and TNFRII-Fc-IL-1ra chimera against recombinant human IL-1's proliferation stimulating activity to D10 cells.

Please replace the paragraph beginning at page 38, line 12 with the following amended paragraph:

Results of cell-based assays are shown in Figures 8-9. Taken together, functional TNFRII-Fc-IL-1ra chimera has been produced successfully. It maintains both TNF alpha and IL-1 neutralizing activity. Due to its mammalian produced nature with glycosylation and large size of the fused molecule, it has longer biological life than TNFRII-Fc (ENBREL Enbrel).

Please replace the paragraph beginning at page 38, line 20 with the following amended paragraph:

125-I labeled TNFRII-Fc-IL-1ra was made by using Iodogen method and purified by size-exclusion chromatography (M Hui et al., 1989). IL-1 receptor binding assay had been established by using in-house mammalian recombinant IL-receptor extracellular domain fused (see Example 3). IL-1 receptor binding to 125-I labeled TNFRII-Fc-IL-1ra was compared side by side with non-radiolabelled TNFRII-Fc (ENBREL Enbrel), and negative control TNFRII-Fc.

Please replace the paragraph beginning at page 39, line 3 with the following amended paragraph:

125-I labeled TNFRII-Fc-IL-1ra was injected into skin inflammation mice model (see below) together with 125-I labeled TNFRII-Fc (ENBREL Enbrel). Surprisingly, our result indicated that

it was distributed more at inflammatory site than that of TNFRII-Fc (Table 3). This most probably is due to its IL-1 receptor binding affinity. Meanwhile TNFRII-Fc was also distributed more at inflammation site with lesser degree than that of TNFRII-Fc-IL-1ra. This may be explained by its TNF alpha binding affinity and high concentration of TNF alpha at inflammation site.

Please replace the table beginning at page 40, line 6 with the following amended table:

Table 2: IL-1 receptor binding to 125-I labeled and non-labeled TNFRII-Fc-IL-1ra (n=3).

Name	Binding OD (X±SD)
TNFRII-Fc-IL-1ra 125-I labeled	1.5±0.3
TNFRII-Fc-IL-1ra	1.5±0.2
TNFRII-Fc (<u>ENBREL</u> Enbrel)	0.4±0.3

Please replace the table beginning at page 40, line 8 with the following amended table:

Table 3: Distribution of 125-I labeled TNFRII-Fc-IL-1ra and TNFRII-Fc (ENBREL Enbrel) in inflamed and non-inflamed skin tissues 4 hours after injection. The distribution is expressed as % of injected dose per gram of tissue (n=6).

Treatment	Tissue	% of injected dose per gram tissue (n=6)
TNFRII-Fc-IL-1ra 125-I	Inflamed skin	3.8±0.2
TNFRII-Fc-IL-1ra 125-I	Normal skin	1.5±0.1
TNFRII-Fc (<u>ENBREL</u> Enbrel) 125-I	Inflamed skin	2.8±0.2
TNFRII-Fc (<u>ENBREL</u> Enbrel) 125-I	Normal skin	1.4±0.2

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Please replace the table beginning at page 40, line 11 with the following amended table:

Table 4: Effect of systemically administration of TNFRII-Fc-IL-1ra, TNFRII-Fc (ENBREL Enbrel), concurrent use of TNFRII-Fc and IL-1ra and negative control Fc fragment during inductive stage on skin inflammation. The skin inflammation is expressed as ear swelling thickness x10-2mm (X±SD)/incidence (% onset of total animal# at day 3)(n=10).

	5ug	10ug	20ug
TNFRII-Fc-IL-1ra	4±0.3/100%	3±0.2/100%	3±0.2/90%
TNFRII-Fc (<u>ENBREL</u> <u>Enbrel</u>)	8±0.2/100%	8±0.2/100%	7±0.2/100%
TNFRII-Fc + IL-1ra	8±0.2/100%	7±0.2/100%	7±0.2/100%
Fc fragment control	16±0.2/100%	15±0.2/100%	16±0.3/100%

Please replace the table beginning at page 41, line 1 with the following amended table:

Table 5: Effect of systemically administration of TNFRII-Fc-IL-1ra, TNFRII-Fc (ENBREL Enbrel), concurrent use of TNFRII-Fc and IL-1ra and negative control Fc fragment during inductive stage on the onset of arthritis. The onset of arthritis is expressed as onset day (X±SD)/incidence (% positive of total animal#)(n=10).

	2.5ug	5ug	10ug
TNFRII-Fc-IL-1ra	27±2/80%	28±2/70%	32±3/70%
TNFRII-Fc (<u>ENBREL</u> <u>Enbrel</u>)	24±2/90%	24±2/100%	24±2/100%
TNFRII-Fc + IL-1ra	24±2/80%	25±2/90%	25±2/100%
Fc fragment control	18±2/100%	19±2/100%	18±3/100%

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Please replace the paragraph beginning at page 30, line 2 with the following amended paragraph:

The invention further provides a method of treating TNF and IL-1 dependent disorders, including administering to a subject in need thereof an effective amount of a composition of the invention. A "TNF and IL-1 dependent disorder" refers to a disorder that is associated with an abnormal level of the gene expression or activity of TNF or IL-1. Examples of such a disease include, but are not limited to, acute and chronic inflammation (e.g., inflammatory conditions of a joint such as osteoarthritis, psoriatic arthritis and/or rheumatoid arthritis); psoriasis; acute hepatitis, cardiovascular diseases, brain injury as a result of trauma, epilepsy, hemorrhage or stroke; and graft versus host disease.